

Effects of Hormone Mimetic Insecticide Tebufenozide on Aquatic Organisms

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ABSTRACT

The effects of the non-steroidal ecdysteroid agonist tebufenozide on larvae of *C. flaviplumus* and *C. riparius* were tested in the laboratory. In the most treatments it reached a statistically significant difference from the control condition. As the concentration of tebufenozide was increased, a relatively larger proportion of the observed mortality was associated with the metamorphosis and molting process. Also, the larval mortality of the first larvae was higher than the fourth-instar larvae in *C. riparius*. In terms of development, the effects of tebufenozide were delayed growth stage in relatively lower concentration such as 10 µg/L treatments. Based on the mortality, the susceptibility followed the first-instar *C. riparius* > the fourth-instar *C. flaviplumus* > the fourth-instar *C. riparius*. The morphological effects showed that the first and second segments of body of exposed larvae with tebufenozide expanded slowly and not made a complete cephalothorax of pupae during long times or days. And the head capsule slipped down and forward, revealing a fragile and non-pigmented, unsclerotized new head capsule. There was obvious significant difference in the male/female ratio in contaminated fourth larvae of *C. riparius*. Also, due to the different emergence periods of male/female in low insecticide concentrations, mating chances or opportunities of insects should be decreased. Accordingly, ecological strategies for keeping of sustainable populations should be disrupted.

INTRODUCTION

Recently, chemicals environmental regulations were effected the disruption of the endocrine system is changing the strategies; in best example, imposex of female molluscs (Matthiesen and Gibbs 1998) and male feminization in vertebrates (Vos *et al.* 2000). The use of invertebrates in risk assessment is complicated by a lack of information of developmental stages and specific endocrine functions. In these points, the test substance, the insecticide tebufenozide (RH-5992), belongs to insect growth regulators, the benzoyl hydrazines was well documented. These substances have been reported to act as agonists of ecdysteroidal molting hormones at the molecular level and cause a variety of hormonal effects in insects and crustacean arthropods (Wing 1988; Clare *et al.* 1992; Dhadialla *et al.* 1998; Retnakaran *et al.* 1995). In selecting a species for this study, *Chironomus riparius* has been extensively used in

environmental assessment schemes and standardized chronic assays (Hill *et al.* 1993; USEPA 1994). An additional test species, *C. flaviplumus* which has been reported a dominant species in organic polluted sites in freshwater streams in Korea was expected similar effects to EDCs. The objective of this study was to investigate the sensitivity of two species midges for detecting endocrine effects of EDCs in aquatic insects.

MATERIAL AND METHOD

Animals were reared in an environmental chamber under long-day conditions with a light: dark cycle of 16 : 8 hours and a light intensity of about 500 lx. Water temperature was constant at $20 \pm 1^\circ\text{C}$ in incubator chamber (Sanyo MIR-553, Japan). The larvae were fed finely grounded fish food (Tetra-Werke, Melle, Germany). A long-day photoperiod was provided to the stock cultures (light: dark = 16 h : 8 h). Individuals of *C. flaviplumus* were collected from the sandy or silt zone of Soktae Stream, organic and anthropogenic polluted stream located in the metropolitan city of the Korea, where it is one of the dominant species.

Tebufenozide (Sigma-Aldrich) used to prepare a stock solution with a nominal concentration of 20 mg/L active ingredient. The half-time of tebufenozide is reported 40 days persistence (Sundaram 1997). As endpoints of the toxicity tests the numbers of emerged adults from each vessel were counted, and emergence accidents and dead pupae were observed. All data were recorded at daily intervals. Rate of dead larvae and emergence data were arcsine square-root transformed prior to one-way ANOVA in order to identify any statistical differences between treatments.

RESULT AND DISCUSSION

In control condition, both the fourth-instar larvae were not a different period of larvae stages. When the concentration of tebufenozide was increased, the period of larvae was decreased in both elder larvae species. Relatively low concentration was affected to delay the development of larval stages in the young and elder larvae of midges. In the most treatments it reached a statistically significant difference from the control group. As the concentration was increased, mortality of *C. flaviplumus* increased but it's of *C. riparius* not dose-dependent manner. That was, mortality of the fourth larvae of *C. riparius* was obvious lower than the fourth larvae of *C. flaviplumus* and the first larvae of *C. riparius*. Based on the mortality of exposed day of each species, the susceptibility followed the first-instar *C. riparius* > the fourth-instar *C. flaviplumus* > the fourth-instar *C. riparius*. General, as the concentration of tebufenozide was high and the life stage of individuals was young, lethal effects promptly exhibited. And the lower concentrations of tebufenozide needed long exposed times for lethal effects of individuals.

There was obvious significant difference in the male/female ratio in the fourth larvae of *C. riparius*. A significant difference was not found the sex ratio in the control treatment: male observed 43% and female was 57%. While treatment groups showed clearly different the emerged male (31–32%) and female (68–69%). Null hypothesis was treatment condition was independent of sex in the test vessels was rejected ($0.025 < P < 0.01$). And therefore subdivided control and treatment conditions, null hypothesis was occurrence of control and treatment condition was independent of sex was rejected ($P < 0.001$). These indicated that tebufenozide treatments affected male/female ratio in fourth-instar larvae of *C. riparius*. The emergence range of male was different female and showed clearly different the overlapping day that was only one day of emergence range for male and female in $10 \mu\text{g/L}$, accordingly it could be reduced mating chances of adult insects.

CONCLUSION

Employing a water-replacement exposure setup, chironomids were subjected to various tebufenozide concentrations. As the concentration of tebufenozide was increased, larvae dead generally dose-dependent manner and a relatively larger proportion of the observed mortality were associated with the metamorphosis and molting process. Many short-period studies suggested no lethal effects organism in low concentration. Although relatively lower concentration such as $10 \mu\text{g/L}$ treatments, the first-instar larvae *C. riparius* were postponed growth phase in this study. And the morphological effects observed some test midges the head capsule slipped down and forward, revealing a fragile and non-pigmented, unsclerotized new head capsule. There was obvious significant difference in the male/female ratio in contaminated fourth larvae of *C. riparius*. The range of emergence day was dose-dependent manner (except male in $10 \mu\text{g/L}$): as the concentration was increased, the emergence range was longer than control.

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